In the name of God

Gold nanoparticles delivered miR-375 for treatment of hepatocellular carcinoma

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www.impactjournals.com/oncotarget/ Oncotarget, 2016, Vol. 7, (No. 52), pp: 86675-86686

Research Paper

Gold nanoparticles delivered miR-375 for treatment of hepatocellular carcinoma

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Keywords: *microRNA, liver cancer, nanomedicine, therapy, AEG-1*

Received: *September 23, 2016* ***Accepted:*** *November 02, 2016* ***Published:*** *November 17, 2016*

Oncotarget

Spotlight

Cision PR Newswire.

The entire **Volume 8 (2017)** is now in PubMed.
Volume 9 (2018) issues (1-59, 61) are now in PubMed. Please see the link.

According to the statement from the MEDLINE reviewers on 6/22/2017, "This journal continues to play a major role in the publication of important basic science research papers. Editorial practices are consistently high. Ethical guidelines are consistently followed. This is an important research journal for the field."

Oncotarget will continue to be indexed in PMC and PubMed.

Impact factor (IF) Web of Science (Clarivate Analytics)

Year	IF	Total Cites
2016	**5.168**	30241
2015	**5.008**	10452
2014	**6.359**	3908
2013	**6.627**	2217
2012	**6.636**	1450
2011	**4.784**	493

Scopus/SJR ranking: 2011-ongoing: Q1 (highest rank). All years Q1 in Medicine and Oncology (subject area).

***Oncotarget* ranks number 1 on Total Documents (2015-2017) among** all journals in Oncology, see here

News: Scopus released 2017 **ratings**. Oncotarget is Q1 (highest rank). It ranks 1 in total documents, with cites per doc (IF equivalent) 4.67

ABSTRACT

MiR-375 is a tumor suppressor miRNA that is downregulated in hepatocellular carcinoma (HCC). However, due to the lack of effective delivery strategies, miR-375 replacement as a therapy for HCC has not been investigated. In the present study, we have developed a straightforward strategy to deliver miR-375 into HCC cells by assembling miR-375 mimics on the surface of AuNPs and forming AuNP-miR-375 nanoparticles. AuNP-miR-375 exhibits high cellular uptake and preserves miR-375's activities to suppress cellular proliferation, migration/invasion, and colony formation, and to induce apoptosis in HCC cells. Furthermore, AuNP-delivered miR-375 efficiently downregulated its target genes through RNA interference. In primary and xenograft tumor mouse models, AuNP-miR-375 showed high tumor uptake, therapeutic efficacy, and no apparent toxicity to the host mice. In conclusion, our findings indicate that AuNPs is a reliable strategy to deliver miR-375 into HCC cells and tissue, and that AuNP-miR-375 has the potential in the clinic for treatment of unresectable HCC.

Introduction

Introduction

- Hepatocellular carcinoma (HCC) is the most common form of liver cancer
- mi RNA → Regulation of gene expression
- Down-regulation of miR-375 has been reported in various tumors
- Hyper methylation of CpG islands in miR-375 promoter region → down-regulation of miR-375 in HCC
- miR-375 suppresses malignant traits of HCC by targeting AEG-1 and ATG7
- Increasing miR-375 expression → decreased HCC cell invasion and proliferation by targeting oncogene YAP1

Introduction

❖ miR375 ⟹ inhibit Akt/Ras induced hepatocarcinogenesis

Nanoparticles for the treatment of cancers

❖ Features of gold nanoparticles:

1- biocompatible

2- easy to be surface functionalize

3- suitable for carrying small molecule and macro molecule drugs

4- ability to enter cells

Introduction

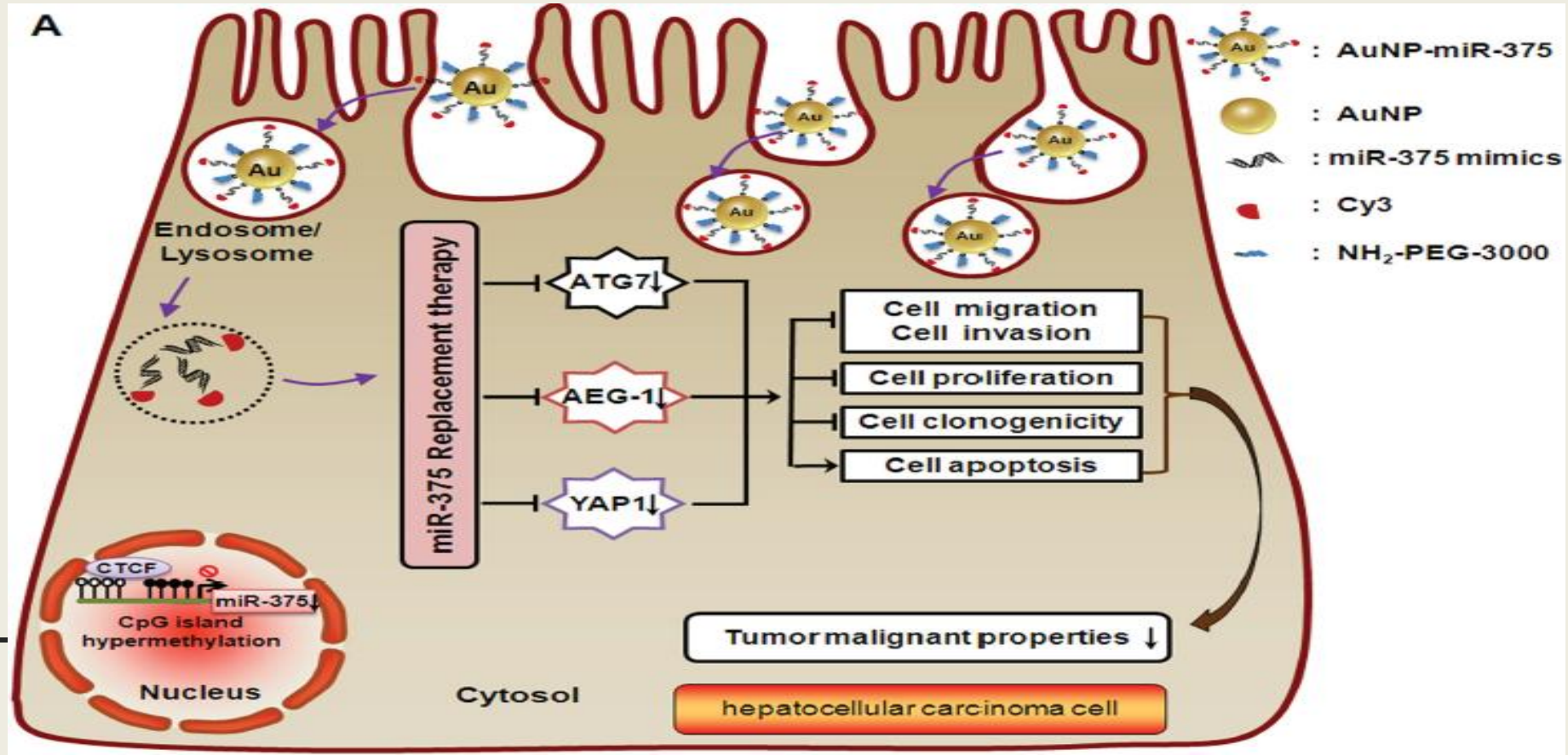
Nanoparticles for the treatment of cancers

- ❖ Oligonucleotides on their surfaces are resistant to nuclease degradation
- ❖ Very low removal by innate immune response
- ❖ Drug carrier and photothermal therapy

Introduction

the purpose of the article

- **Prepare an AuNP system to deliver miR-375 for miRNA Replacement therapy in HCC**



Methods

Methods

- ❖ Fluorescence imaging
- ❖ Transmission electron microscope(TEM)
- ❖ Zeta sizer(DLS/ Zeta potential)
- ❖ Uv-vis spectroscopy
- ❖ Taq-Man qRT- PCR
- ❖ Cell counting kit8(CCK-8)
- ❖ Wound-healing assay
- ❖ Colony formation assay
- ❖ Immunochimestrary
- ❖ Bio luminescence
- ❖ TUNEL Assay
- ❖ Flow cytometry
- ❖ Western blot
- ❖ Matrigel invasion assay
- ❖ Synthesis of citrate-coated gold nanoparticles (Frence Method) and AuNP-miR-375
- ❖ histological examination
- ❖ immunostaining

Result and methods

Result and methods

❖ Preparation and characterization of AuNPs and AuNP-miR-375

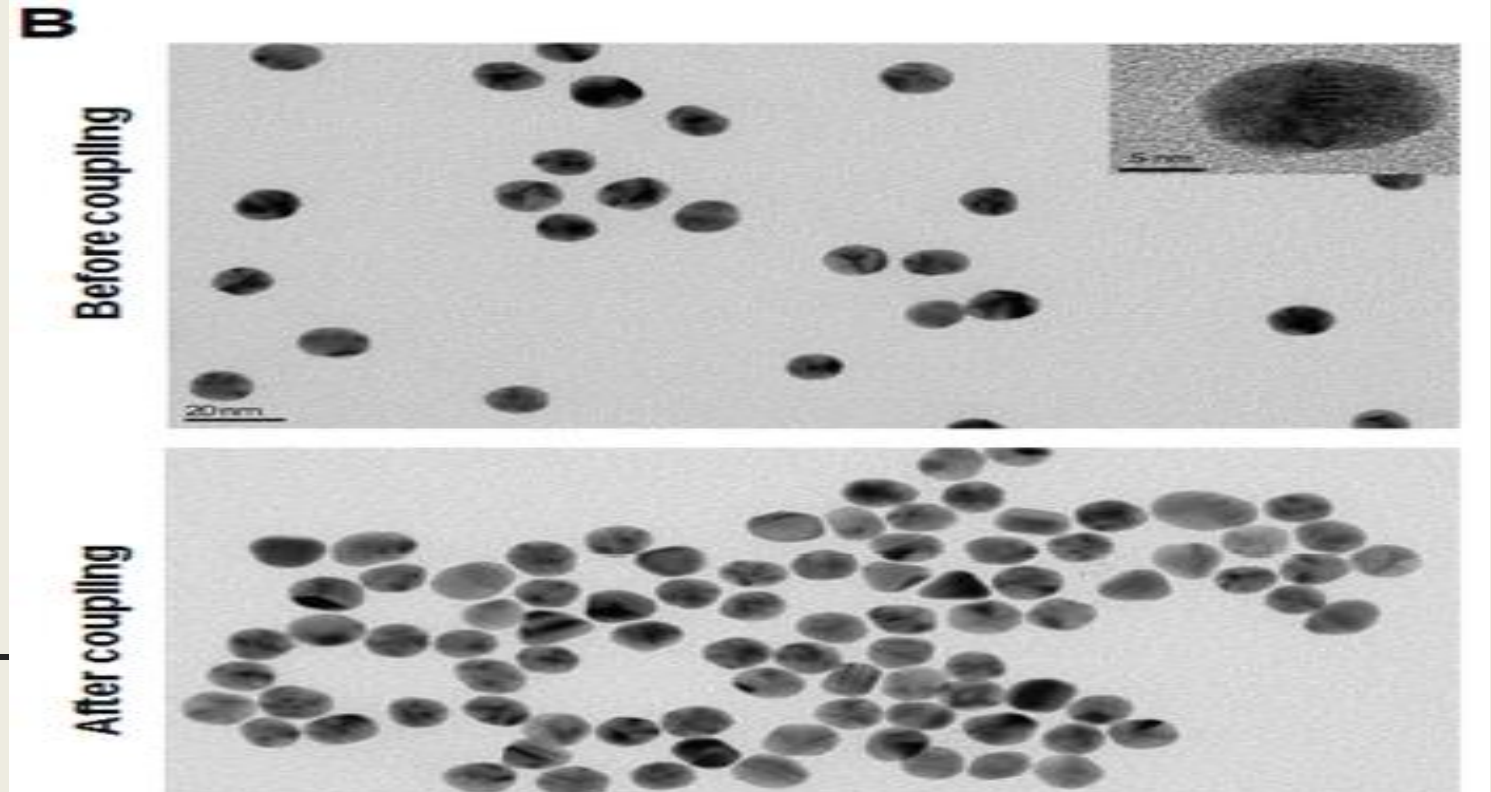
- ❖ **AuNP were synthesized by using France method (Reduction of golden salt with sodium citrate salt)**
- ❖ **Two-strand miR-375 mimics ⟹ Cy3 at the end of the antisense strand**
- ❖ **miR-375 ⟹ linked to AuNPs conalently through a gold- sulfur bond**
- ❖ **A PEG layer on the particle surface was used to stabilize AuNP-miR-375**

Result and methods

❖Compare the features of AuNP and AuNP-miR-375

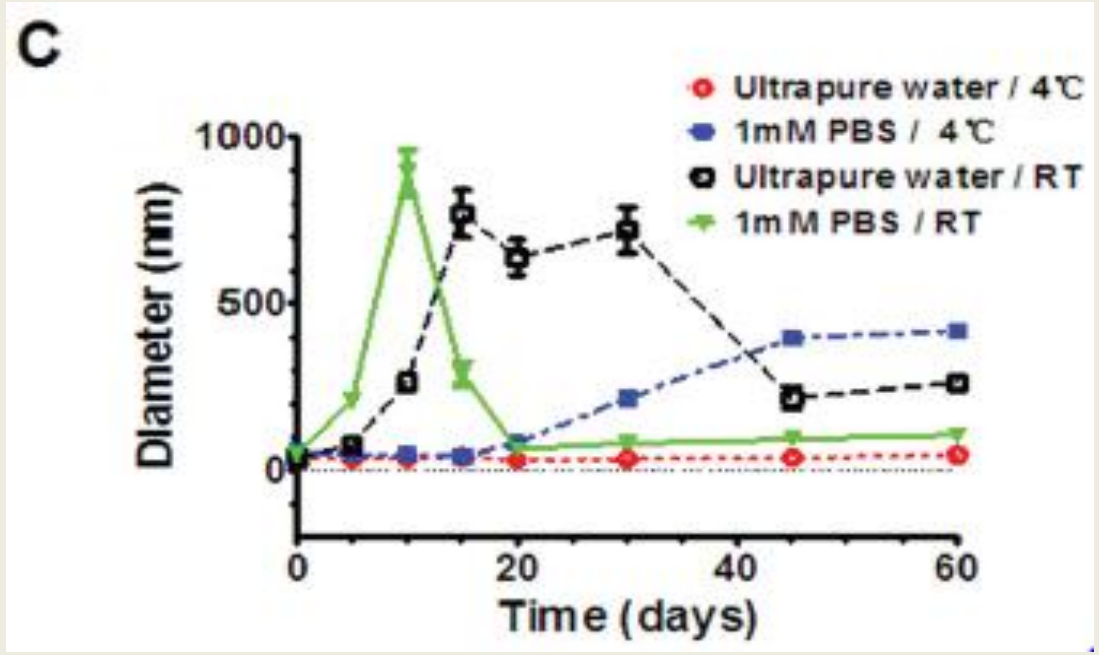
Table 1: Characteristics of AuNPs determined by dynamic light scattering (DLS)

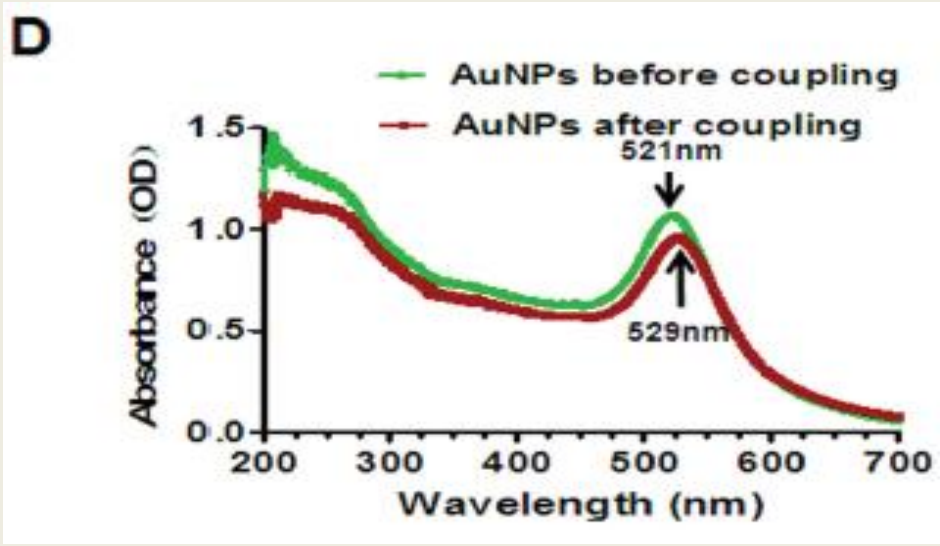
Groups	Particles size (nm)	Polydispersity	Zeta potential (mV)
AuNPs	36 ± 3	0.197 ± 0.034	- 55 ± 0.7
AuNP-miR-375	53 ± 8	0.321 ± 0.191	- 34 ± 1.8



Result and methods

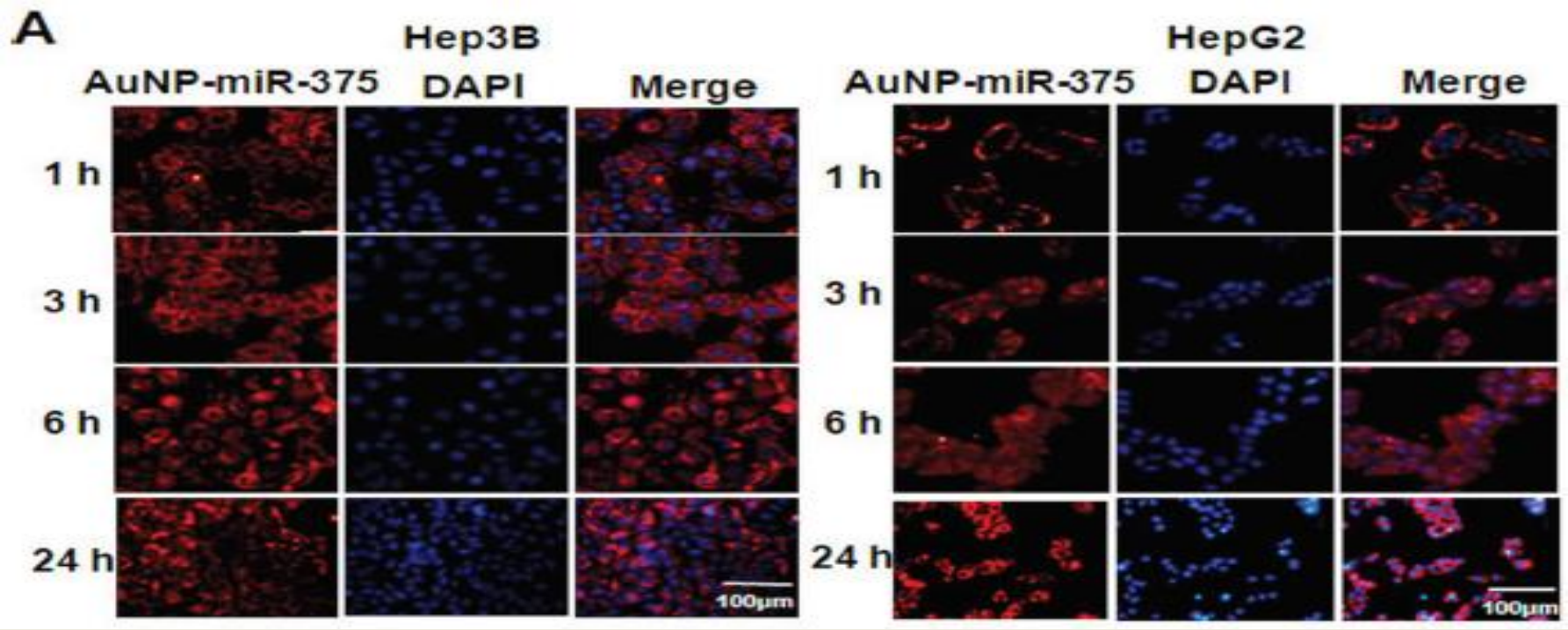
❖Compare the features of AuNP and AuNP-miR-375





Result and methods

- **cellular uptake of AuNP-miR-375 and release of miR-375 in hepatoma cells**
- **Check cellular uptake of cy3-labeled AuNP-miR-375 by fluorescence microscopy and by flow cytometry analysis**

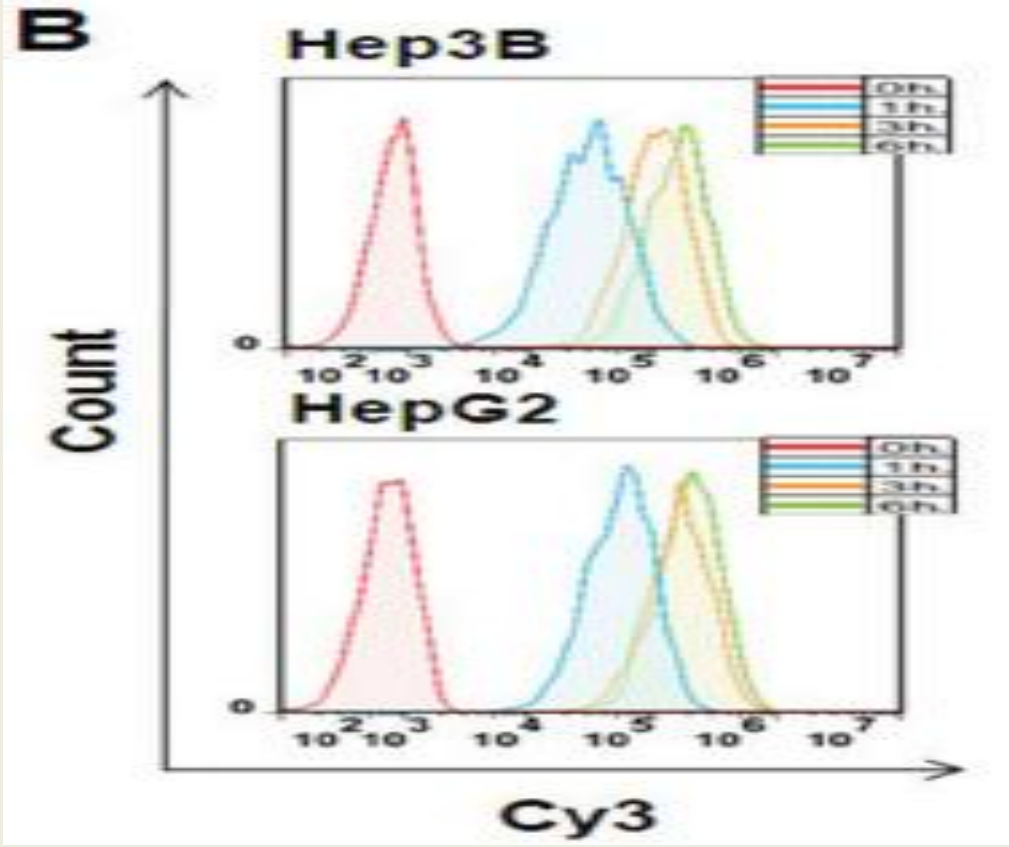


Result and methods

- **cellular uptake of AuNP-miR-375 and release of miR-375 in hepatoma cells**

- **Check cellular uptake of cy3-labeled AuNP-miR-375 by flow cytometry analysis**

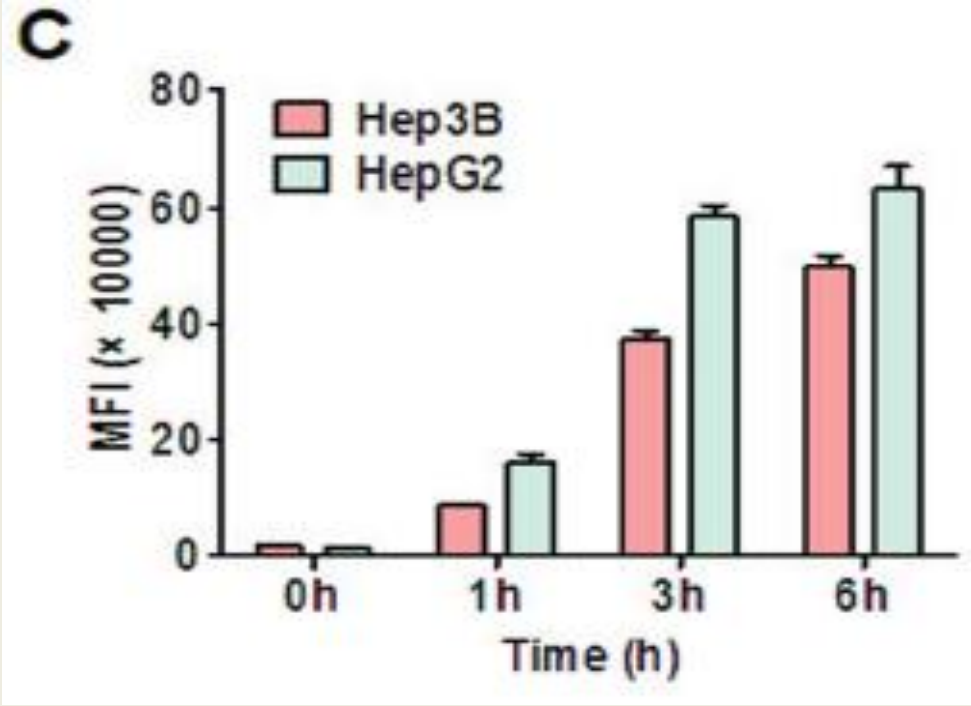
quantify the fraction of cells taking up AuNP-miR-375



Result and methods

- **cellular uptake of AuNP-miR-375 and release of miR-375 in hepatoma cells**

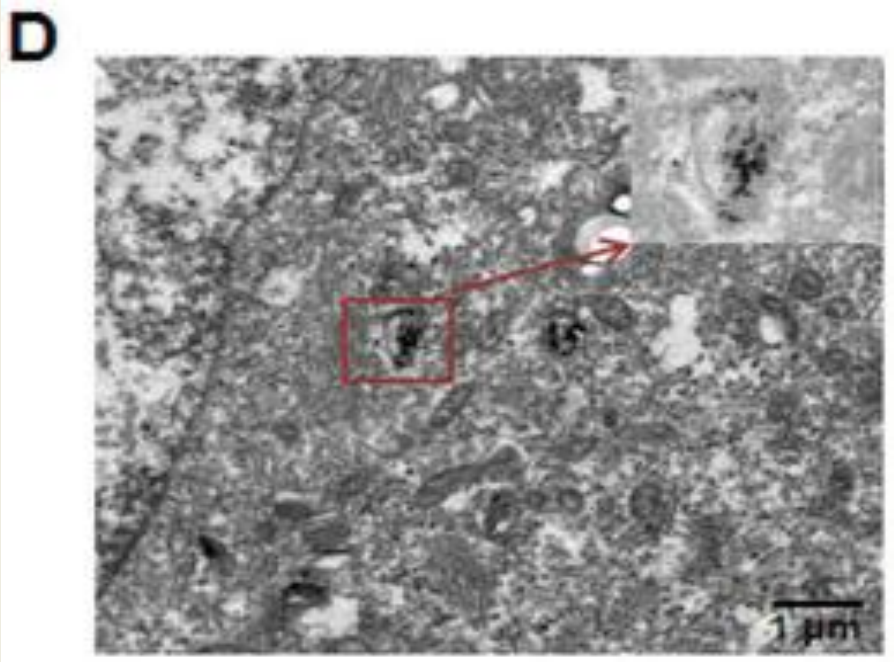
- **Saturation of cells**



Result and methods

- **cellular uptake of AuNP-miR-375 and release of miR-375 in hepatoma cells**

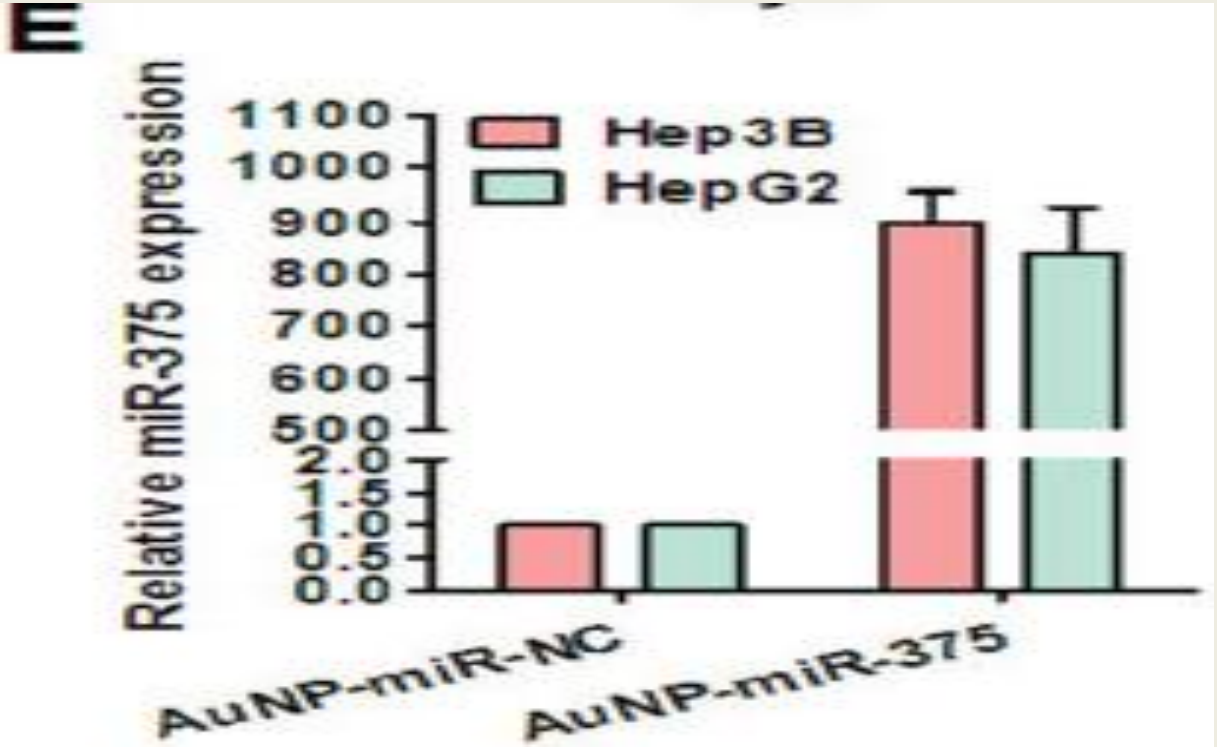
- **The location of AuNP-miR-375 in the cell**



Result and methods

- **cellular uptake of AuNP-miR-375 and release of miR-375 in hepatoma cells**

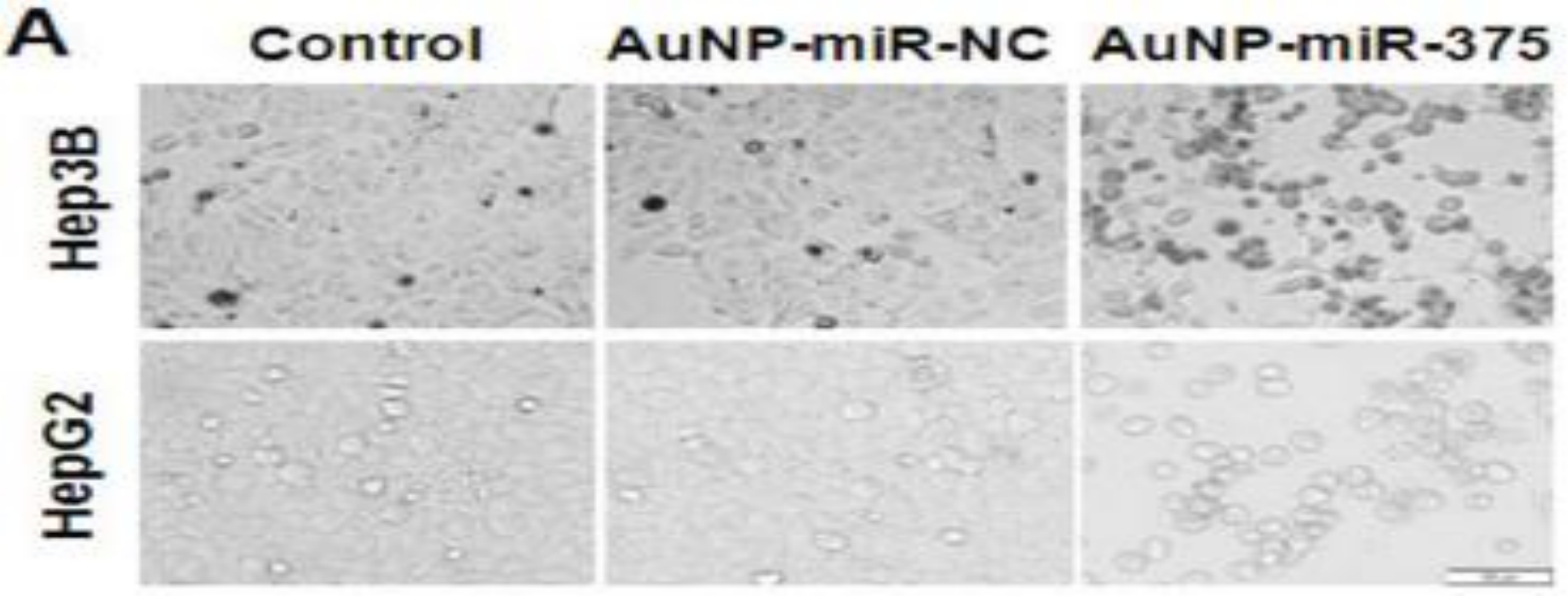
- **Use Taq Man qRT-PCR to detect release of mature miR-375 in hepatoma cells**



Result and methods

❖ AuNP-miR-375 suppresses tumor cell phenotype in vitro

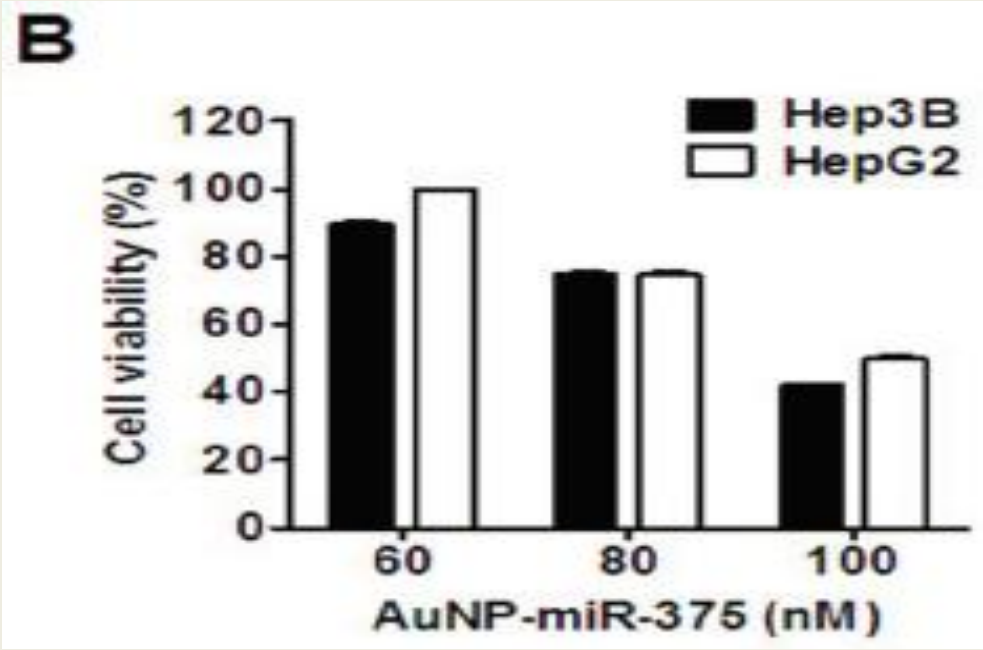
❖ AuNP-miR-375 induce significant cell growth inhibition and cell death in HepG2and Hep3B



Result and methods

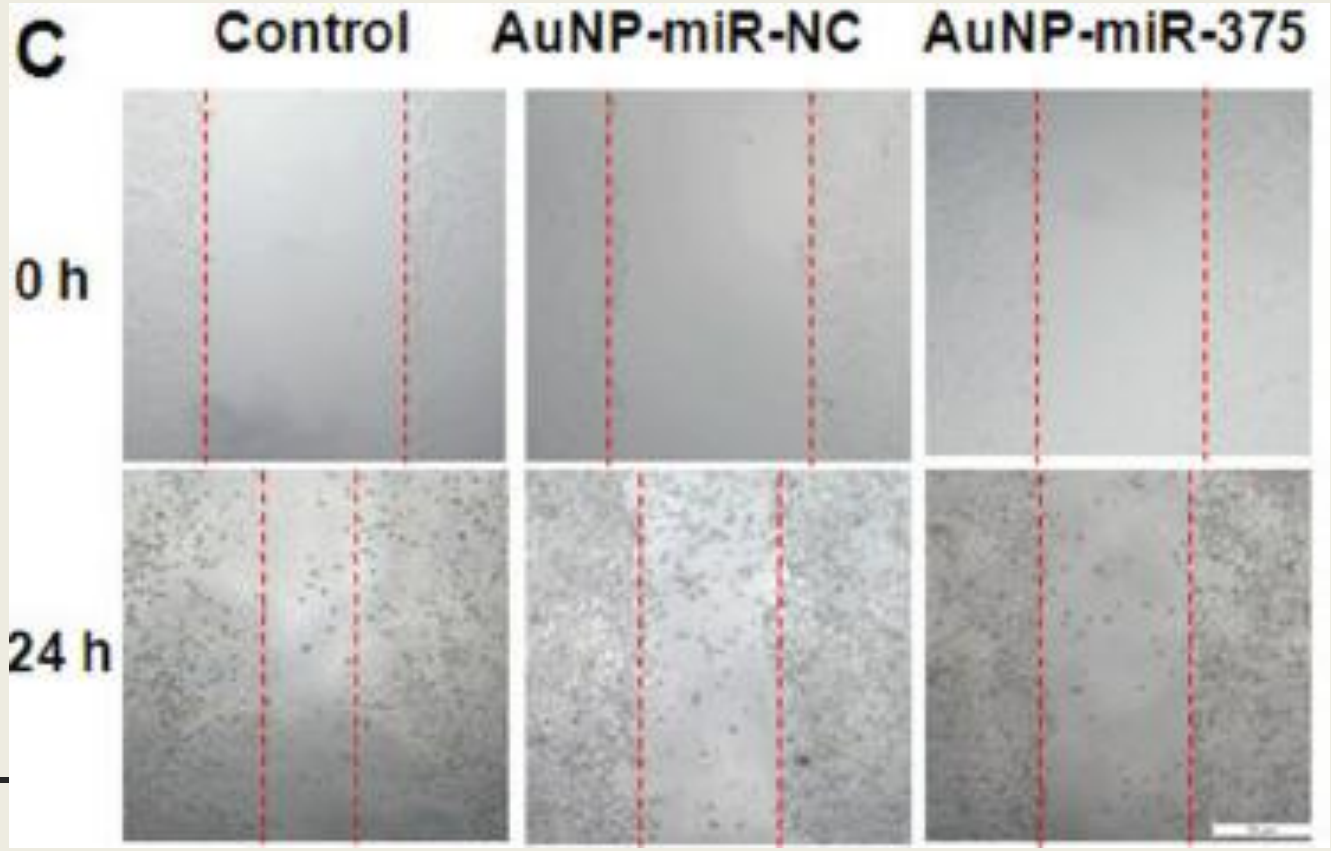
❖ AuNP-miR-375 suppresses tumor cell phenotype in vitro

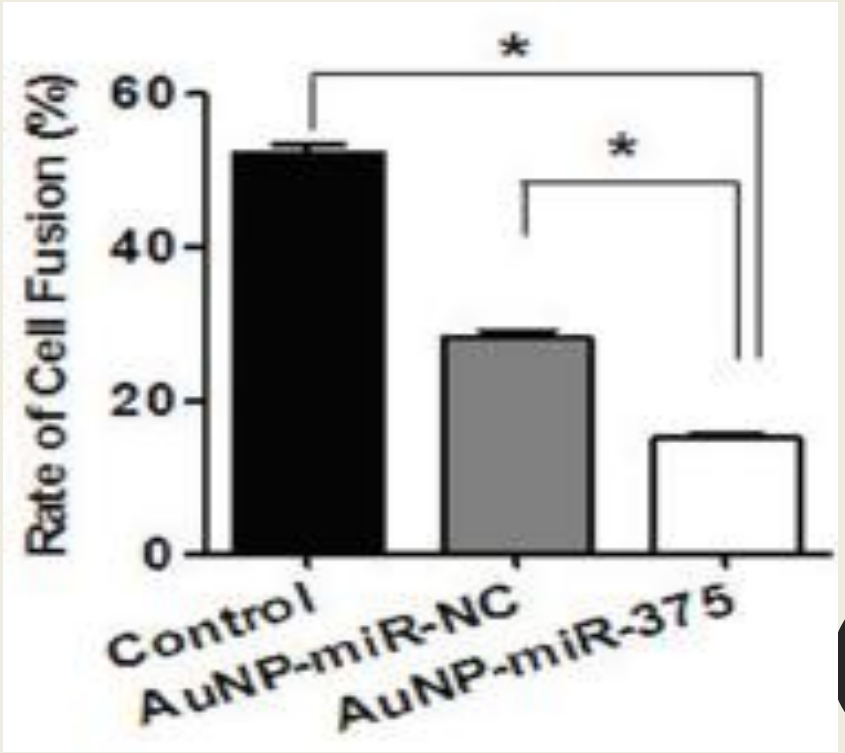
❖ Measure cell proliferation with various concentration of AuNP-miR-375 (CCK-8)



Result and methods

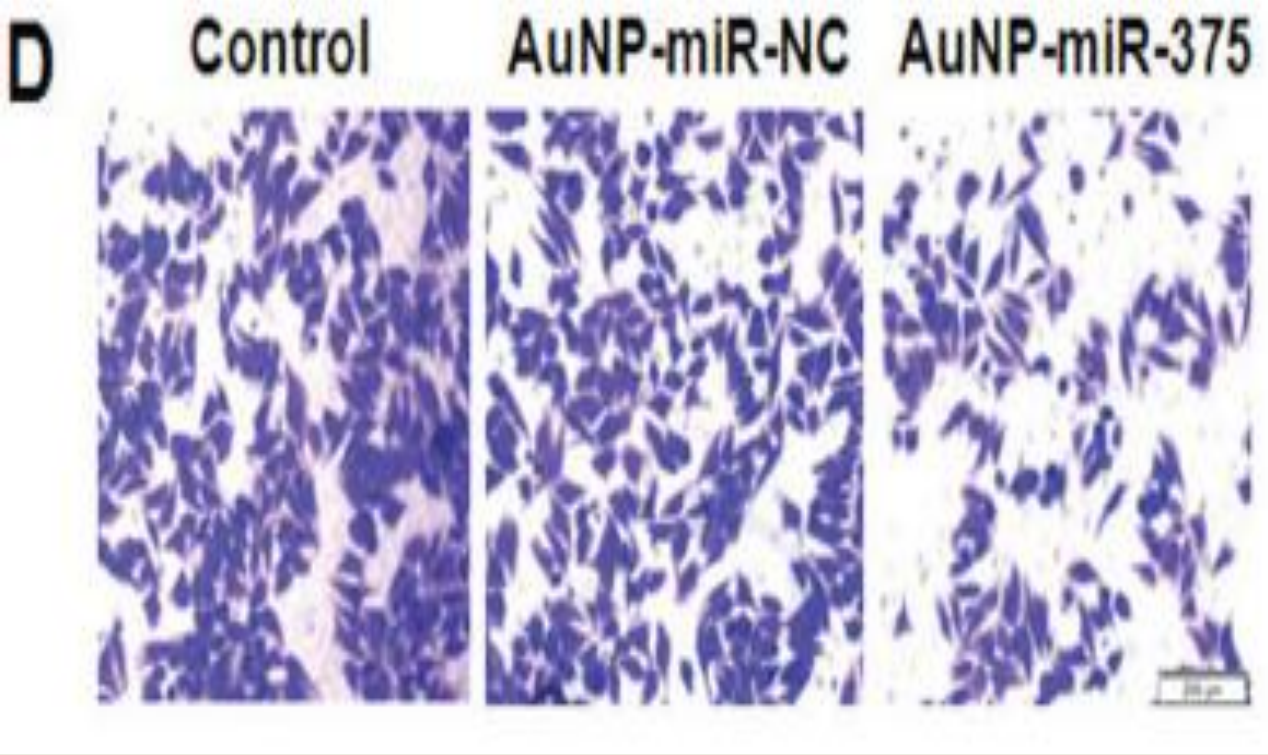
- **AuNP-miR-375 suppresses tumor cell phenotype in vitro**
- **check the effect of AuNP-miR-375 on motility of HCC**
- **Wound – healing assay**

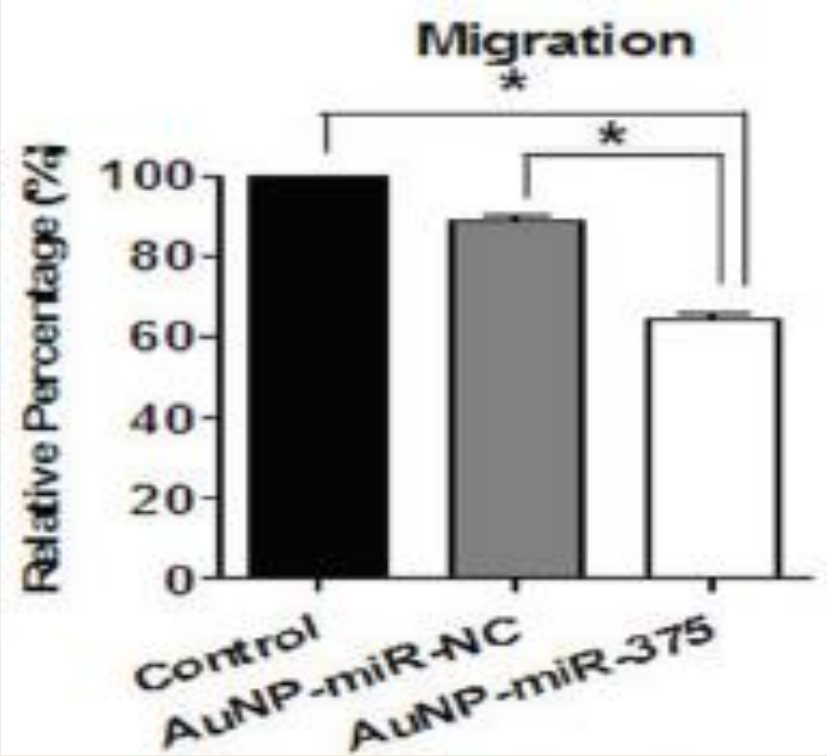




Result and methods

- **AuNP-miR-375 suppresses tumor cell phenotype in vitro**
- **check the effect of AuNP-miR-375 on motility of HCC**
- **Trans well assay**



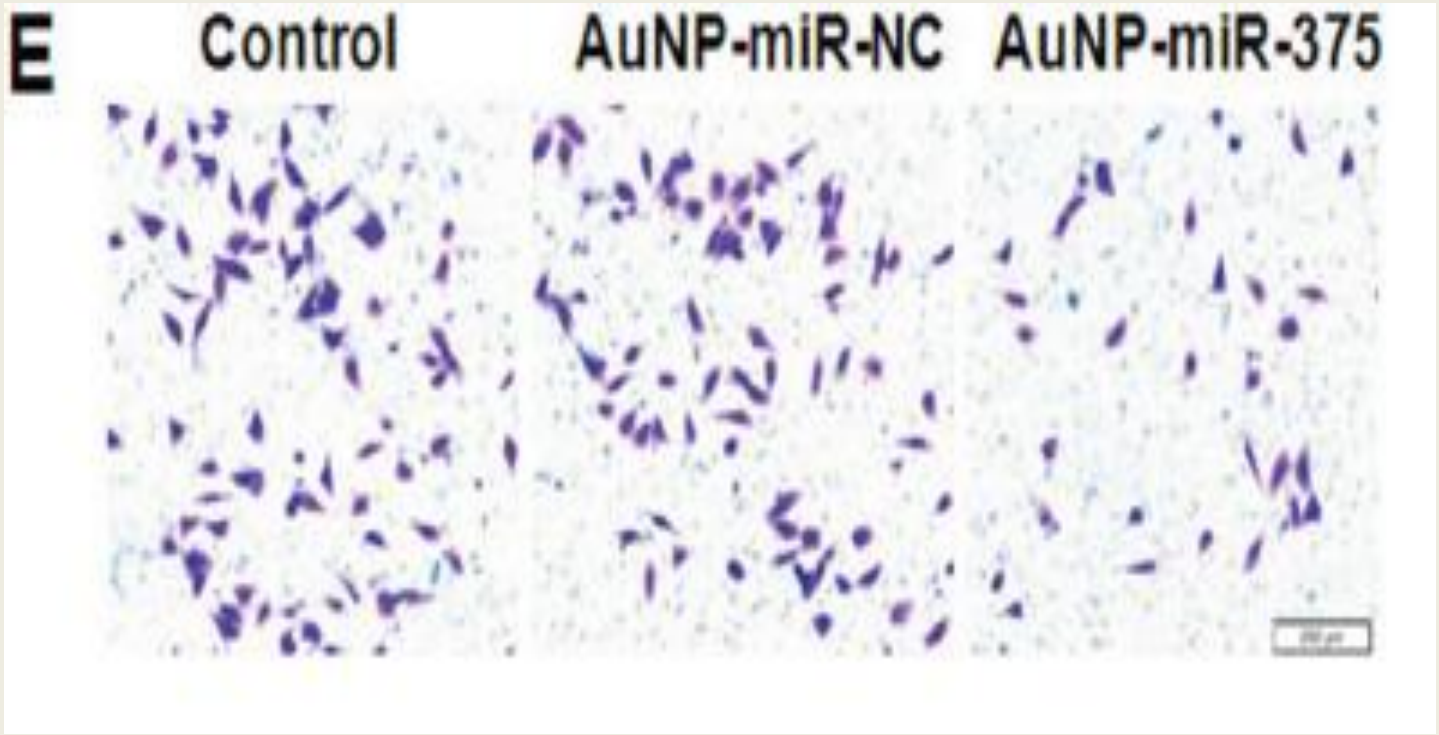


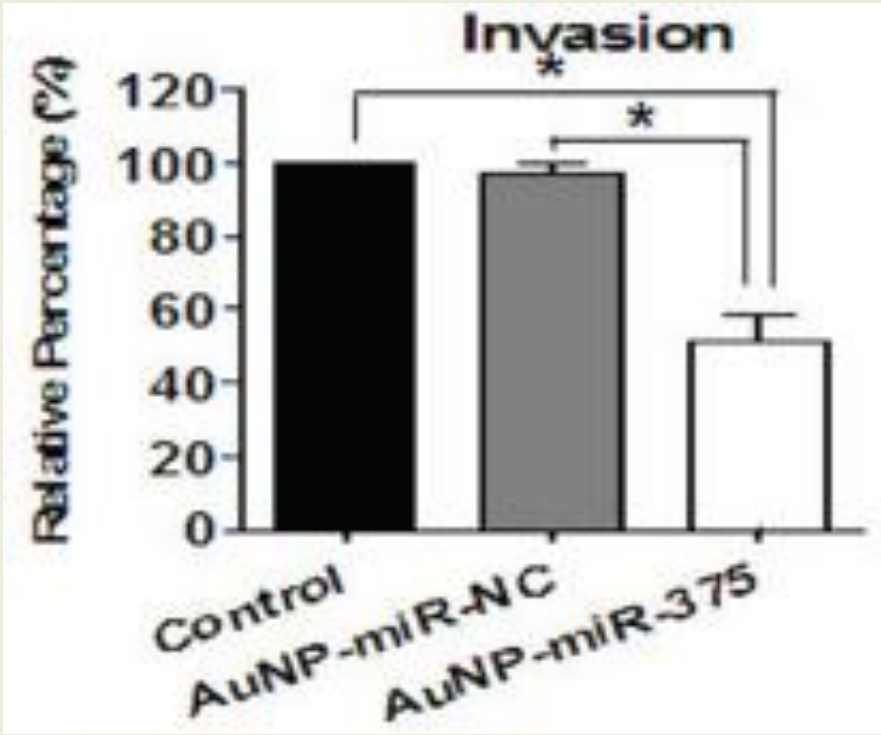
Result and methods

❖ AuNP-miR-375 suppresses tumor cell phenotype in vitro

❖check the effect of AuNP-miR-375 on motility of HCC

❖ Matrigel invasion assay

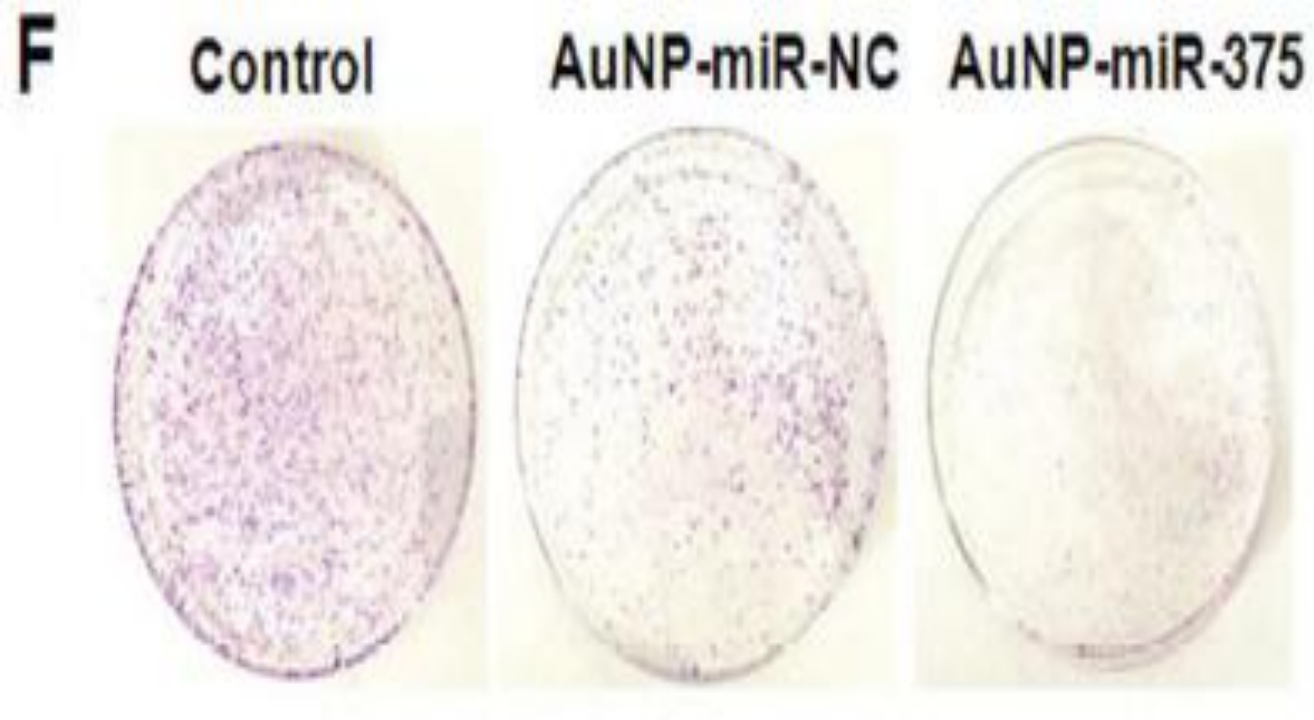


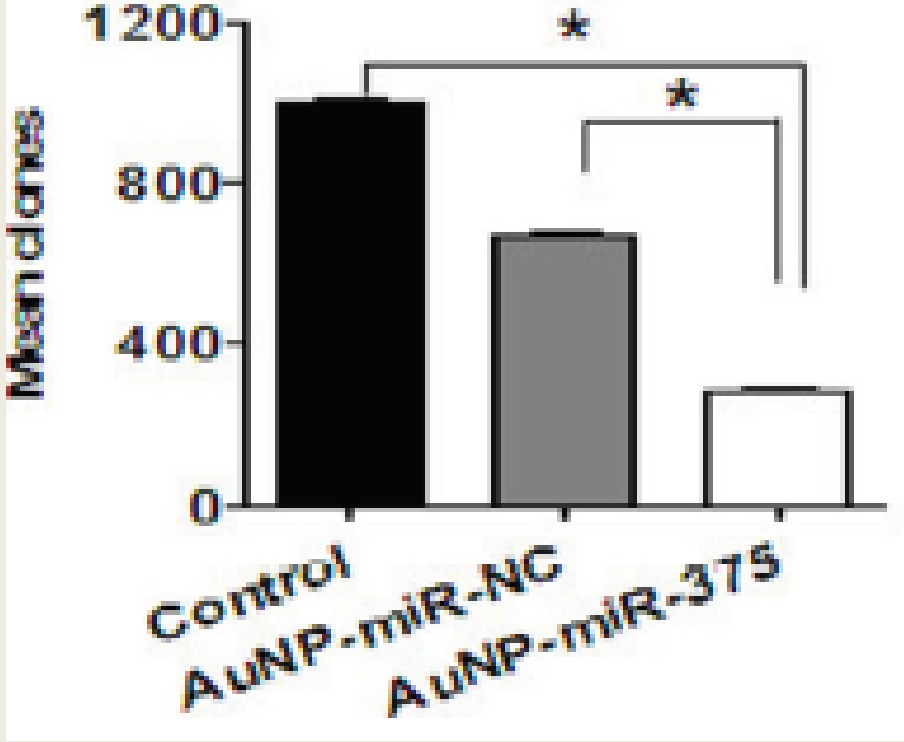


Result and methods

❖ AuNP-miR-375 suppresses tumor cell phenotype in vitro

❖ Study of cell proliferation by colony formation assay

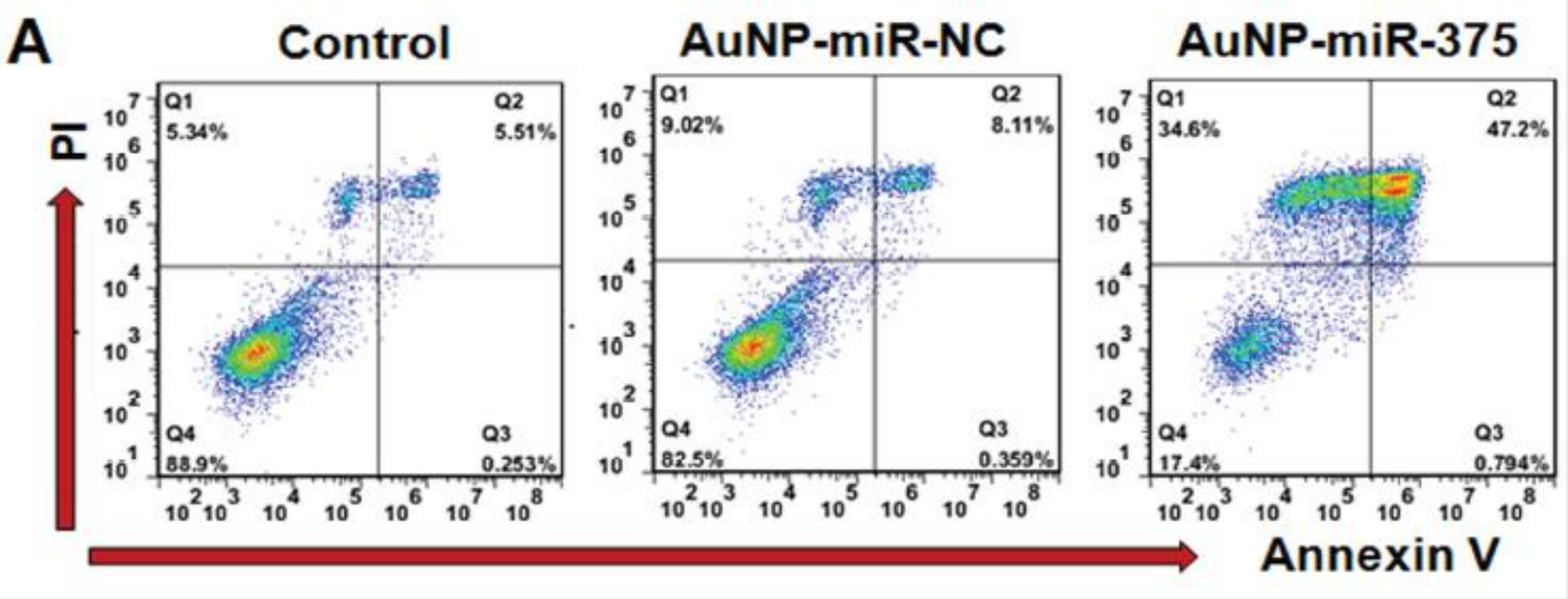




Result and methods

❖ AuNP-miR-375 suppresses tumor cell phenotype in vitro

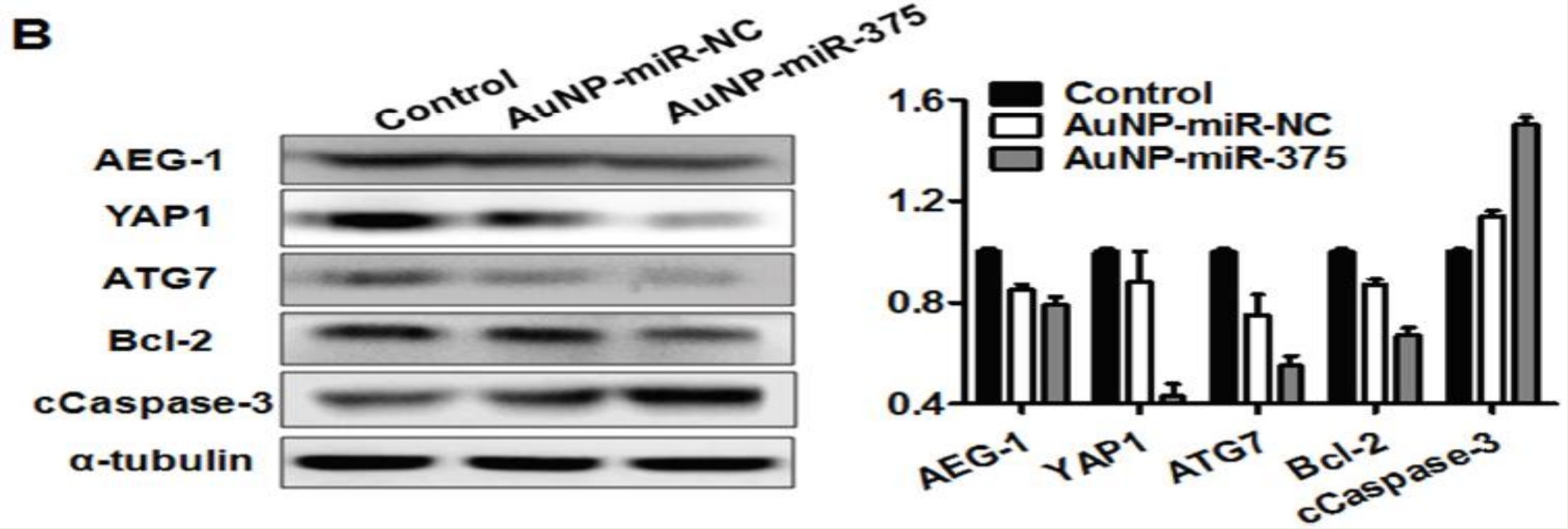
❖ Study of apoptosis by flow cytometry



Result and methods

❖ AuNP delivered miR-375 can act on its downstream gene targets

❖ Check expression of miR-375 downstream gene targets in Hep3B cells treated with AuNP-miR-375 (with western blot)

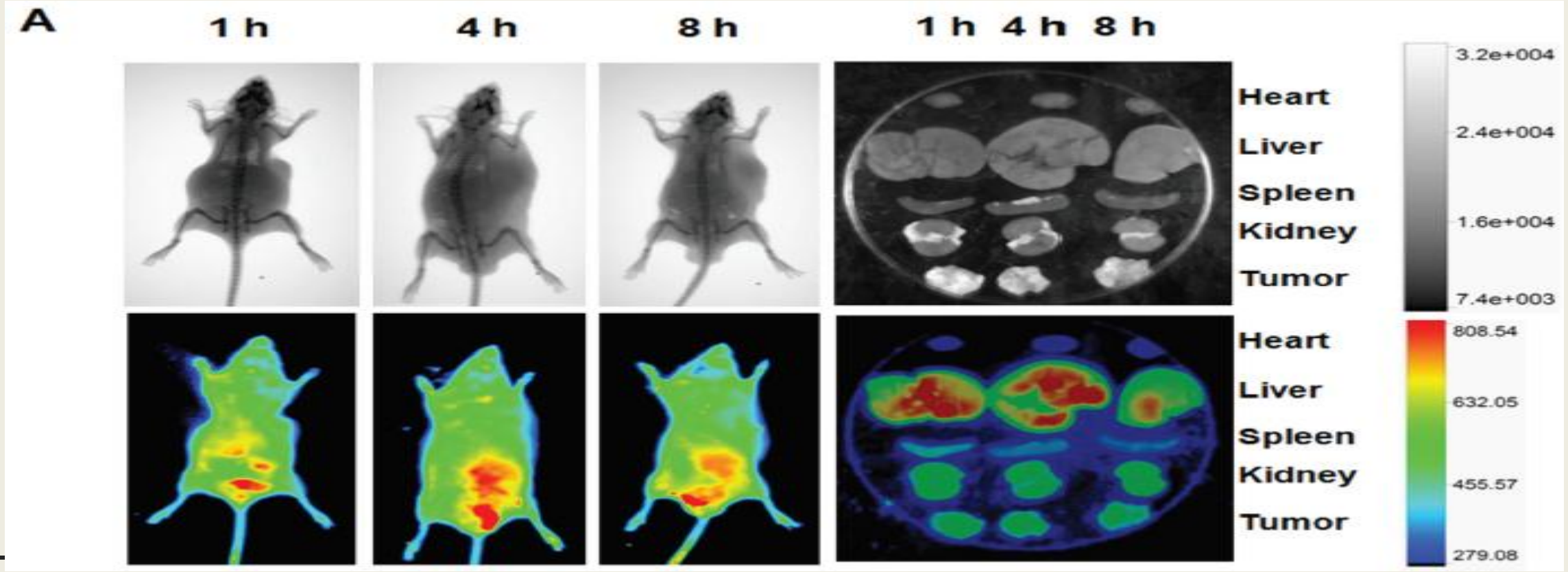


Result and methods

❖ tissue distribution and toxicity of AuNP-miR-375

❖ **Study of Effective and specific transport of miR-375 to tumor tissue by AuNP-miR-375**

❖ **Study of distribution of cy3-labeled miR-375 in tumor bearing BALB/c nude mice following i.v. administration**

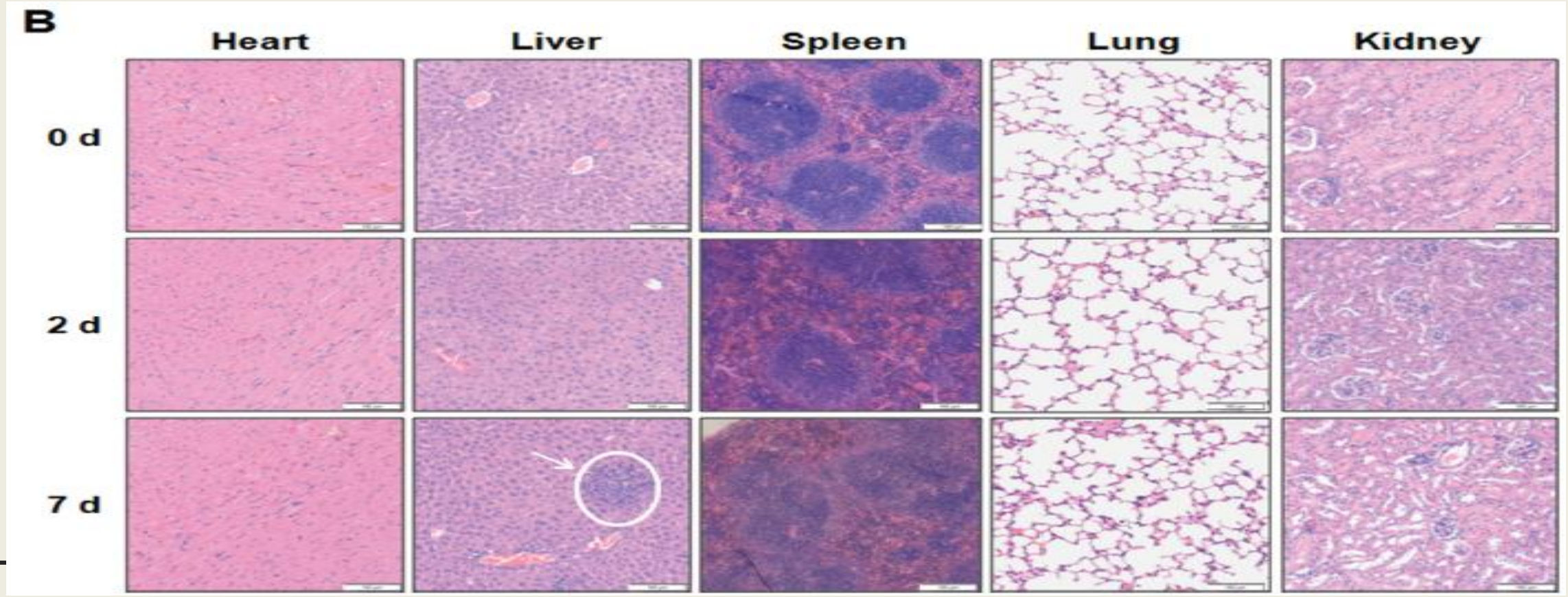


Result and methods

❖ tissue distribution and toxicity of AuNP-miR-375

❖ **Evaluate the safety of AuNP-miR-375**

❖ **AuNP-miR-375 injection into the mice intravenously through the tail vein**

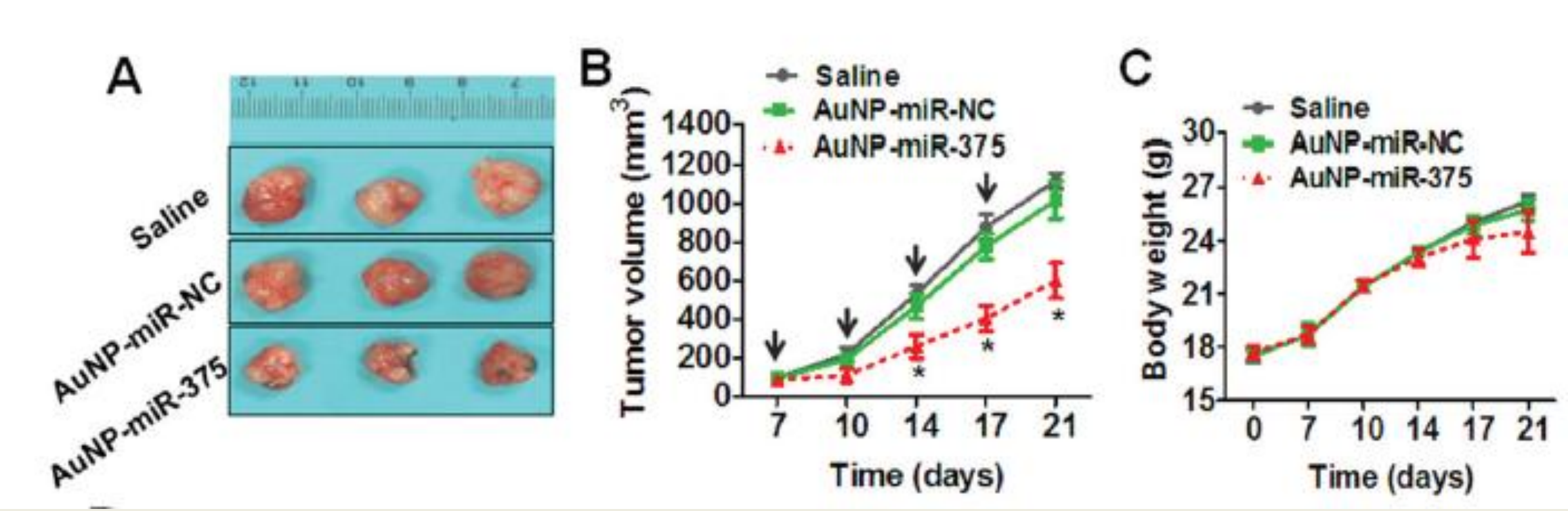


Result and methods

❖ AuNP-miR-375 suppressed tumor growth in primary and xenograft tumor mouse models

❖ Study of Anti-tumor effect of AuNP-miR-375 on HepG2 xenograft tumor mouse model

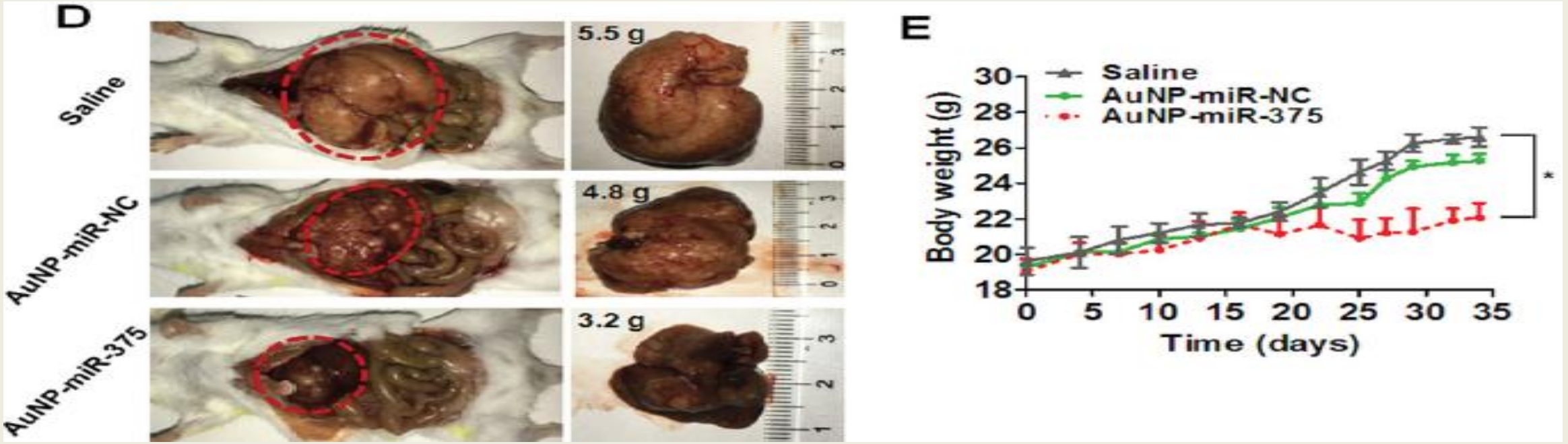
❖ Inoculation of 5×10^6 HepG2 cells in the front armpit of BALB/c nude mice



Result and methods

❖ AuNP-miR-375 suppressed tumor growth in primary and xenograft tumor mouse models

- ❖ Study of therapeutic activity of AuNP- miR – 375 in primary HCC tumor
- ❖ Hydrodynamic injection of plasmid carrying two gene (AKt / Ras) in to mice

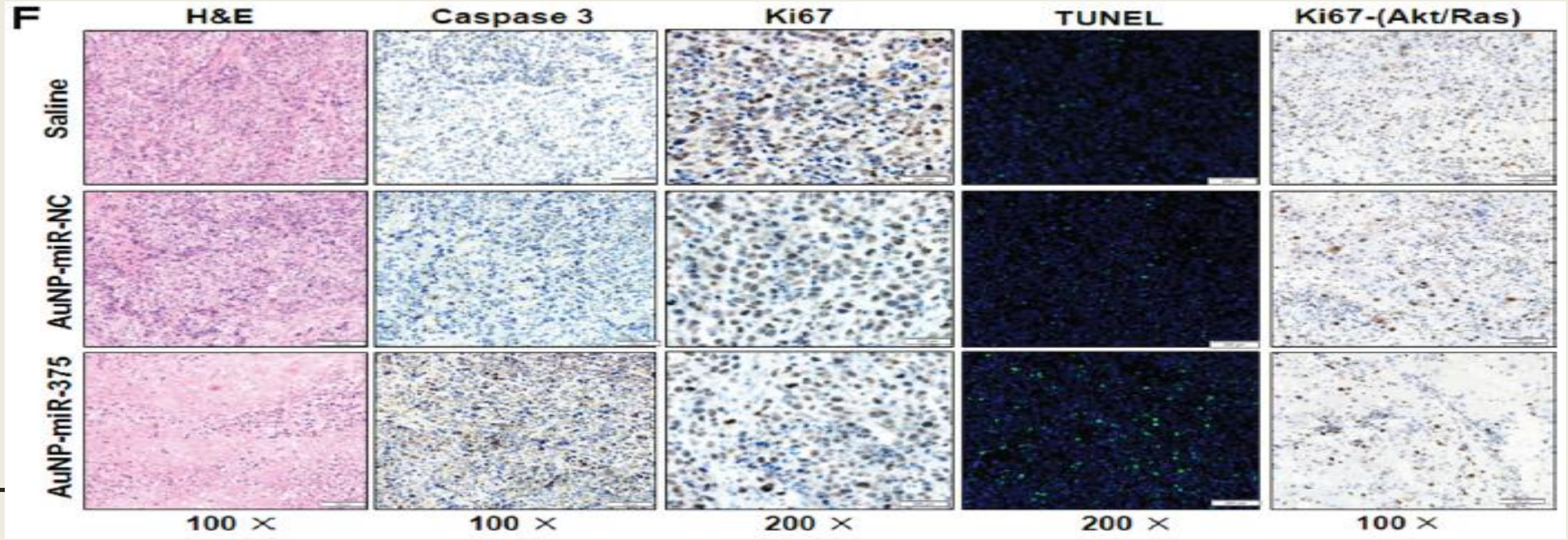


Result and methods

- **AuNP-miR-375 suppressed tumor growth in primary and xenograft tumor mouse models**

- **1- histological examination 2- TUNEL assay 3-immunohistochemical analysis**

  4- immunostaining



Discussion

Discussion

- **miR-375 ⟹ important tumor suppressor in HCC**
- **the lack of an efficient delivery system has been a major obstacle impeding the therapeutic application of miR-375 in HCC**
- **In this study ⟹ AuNP-miR-375 delivery system to deliver miR-375 in to HCC cells and tissues**
- **AuNP- miR – 375 ⟹ can enter HCC cells or tissues and function as a tumor suppressor**
- **AuNP-miR-375 has same biological function as endogenous miR-375**
- **AuNP-miR-375 ⟹ restore the regulation networks of miR-375**

Discussion

- **Previous research → overexpression of miR-375 in hepatoma cells by transfection of miR-375 precursor with lipofectamine 2000 inhibited tumor growth**
- **AuNP-miR-375 can overcome physiological obstacles and deliver miR-375 in to HCC tissues in mice**
- **Highly distributed AuNP-miR-375 in HCC tissues**
- **AuNP can serve as an attractive platform for nucleic acid delivery in cancer treatment**
- **AuNP are biocompatible and non-toxic**

Discussion

- **AuNP are easy modified to deliver nucleic acid**
- **High uptake efficiency and release of intact miR- 375**
- **AuNP → protect mi RNA from degradation**
- **AuNP it self possess anti- tumor effect and may improve the efficacy of delivered anti- tumor agents**

Conclusion

conclusion

- **In conclusion, in this study illustrated the reliability of AuNPs to deliver miR-375 into HCC cells and the therapeutic effects of AuNP-miR-375 in HCC treatment**

Future perspective

Future perspective

- **Excellent stability and biocompatibility of AuNPs displayed in this study warrant more extensive investigation of its application in delivery miRNA or siRNA. Our study also highlights the therapeutic potential of miR-375 in HCC treatment and support the development of more effective therapeutic strategies that target miR-375 (or other dysregulated miRNAs) by nanotechnology.**